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PRINCIPAL INVESTIGATOR: Peter J. Thraves, Ph.D.

CONTRACTING ORGANIZATION: Georgetown University
Washington, DC 20007

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Peter J. Thraves, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Dept of Radiation Medicine, Division of Radiation Research
Georgetown University Medical Center
TRB E204, 3970 Reservoir Road NW-Washington DC 20007

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13. ABSTRACT

In this study we have shown that ionizing radiation can transform immortalized/ initiated human breast epithelial cells from a donor with Li-Fraumeni syndrome (LFS) containing a germline mutation in the *p53* gene to a malignant phenotype. Exponentially growing human breast cells (HME-50) were irradiated with 2 Gray of gamma radiation with additional doses of 2 Gray delivered at daily intervals to a total dose of 60 Gray. After each increment of 10 Gray the cultures were allowed to recover for 10 7-10 days. After this recovery the cultures were tested for changes in morphology, anchorage-independent growth, growth-factor requirements, growth in the presence of serum and tumor formation in *scid* mice. In comparison to cultures which were unirradiated or treated with total dose of 10 and 20 Gray, HME-50 cells treated with 30-60 Gray exhibited distinct changes in cellular morphology, reduced growth factor requirements, increased cell density at confluence and anchorage-independent growth. Most significantly, they reproducibly produced tumors in *scid* mice at a high frequency. Further studies using clonal isolates from these radiation transformed breast cells will be required to determine the role of the remaining wild-type *p53* allele in this transformation process.

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ORIGINAL STATEMENT OF WORK

The proposed studies were designed to test the hypothesis that ionizing radiation (x-rays) can promote the development of malignant cells from immortalized human breast epithelial cells isolated from a donor with Li-Fraumeni's syndrome, which contain a mutant p53 tumor suppressor gene. Further, they were designed to determine if the remaining wild-type alleles were targets for the mutagenic effects of ionizing radiation in this type of transformation.

YEAR 1

Radiation treatment of immortalized human breast epithelial cells containing p53 gene mutations with repeated doses of x-rays (2Gy).Cellular characterization of radiation transformed human breast cells: morphology (focus-formation), growth characteristics (growth factor independence), anchorage independent growth (soft agar colony formation),and tumorigenicity (tumor formation in athymic mice).Chromosomal and isoenzyme analysis.

YEAR 2

Determine the role of p53 genes in radiation-induced transformation of human breast epithelial cells.The expression of p53 will be determined by Western blotting,immunoprecipitation with specific antisera and RNA analysis.Allelic loss,gene rearrangements and additional mutations will be determined by restriction enzyme analysis,single-strand conformational polymorphism analysis (SSCP) and DNA sequencing.

FINAL REPORT

The preliminary evidence relating to the investigation of this hypothesis includes:

1. Epidemiological studies have shown that individuals with germline mutations of the *p53* gene have an increased incidence of breast cancer (21-23).
2. X-rays have been shown to transform normal human breast epithelial cells to a malignant phenotype (40). This transformation involved the loss of normal *p53* protein due to the deletions in both wild-type alleles following radiation exposure.
3. An immortalized human breast epithelial cell line (HME50) from a donor with Li-Fraumeni's syndrome, containing a germline *p53* mutation has been established as model system for the study of *p53* (103).
4. We have previously shown that multiple doses of x-rays will transform immortalized human keratinocytes to a malignant phenotype (38).

Our prior experience with the human epidermal keratinocyte cell system has provided a basis and an important comparison for these studies with human mammary epithelial cells, (HME50). The data we present demonstrates the ability of multiple doses of gamma rays to induce changes at the cellular level during the induction of malignant phenotypes in human breast epithelial cells. These studies were performed to develop a model system for the study of radiation-induced transformation in a human breast epithelial system.

Human breast cells (HME50 and HME32, an immortalized clone from a normal donor) were irradiated using a protocol similar to that used in the therapeutic treatment of breast carcinoma *in situ*. The radiation toxicity studies shown in Figure 1 demonstrate that a dose of 2 Gray gives an approximate survival rate of 90%for HME50 cells. A similar radiation toxicity profile was seen with the HME32 cells derived from a normal donor. This dose (2Gray)

was used as the standard treatment dose for both cell lines (HME50 and HME32) during the entire study. Exponentially growing HME50 and HME32 cells were inoculated at 5 x 10^5 per 75 cm^2 flask and irradiated 24 hours later with 2 Gray of gamma radiation using a JL Shepard Mark II Cesium 137 irradiator at a dose rate of 2.37 Gray/min. Additional doses of 2Gray were delivered at daily intervals to a total dose of 60 Gray was achieved. After each cumulative 10 Gray increment, the cultures were allowed to recover for 7-10 days, Figure 2 . After this recovery, a portion of the irradiated cultures were tested for changes in morphology, anchorage-independent growth, growth factor requirements (i.e. removal of BPE, EGF or insulin), growth in presence of serum and tumorigenicity. The remaining cells were used for the additional radiation treatments to a total dose of 60 Gray. The radiation protocol is outlined in Figure 2 .

We observed no changes in either morphology or growth to saturation density in the unirradiated HME50 cultures, Figure 3A and B or those treated with cumulative doses of 10 or 20 Gray, Figure 3C. These cultures remained flat with a polygonal morphology and saturation densities of approximately 2 x 10^5 cells per cm^2. In contrast, the HME50 cultures treated with 30, 40 and 60 Gray, (50 Gray data not shown), showed significant and increasing morphological changes with increasing dose. Figure 3D-F. The changes in morphology included focal development while at confluence and the piling-up of cell on the monolayer of contact-inhibited cells. These cultures were subcultured to enable potential transformed cells to outgrow and replace the non-transformed cells. Subsequently, cells from these cultures were seeded at 3 x 10^3 per 100 mm dish for saturation density studies. HME50 cells from the culture irradiated with 30-60 Gray of gamma rays showed a 2-3 fold increase in the number of cells at confluence, Table 1. In particular, morphological transformation and increases in saturation density (3-4 fold) at confluence have been cultures treated with 30-60 Gray exhibited anchorage-independent growth, Table 1. No such cellular changes were observed in either the irradiated or unirradiated HME32 cells during identical treatments with radiation performed in a parallel study.

We then tested human breast cells (HME50 and HME32) treated with 10,20,30,40,50 and 60Gy of gamma-rays for tumor formation and anchorage-independent growth. Several of the HME50 cultures produced both of these phenotypic changes. In contrast, HME32 cultures whether irradiated or not, did not show any phenotypic changes. The development of the anchorage-independent phenotype following radiation treatment was also determined (109). Human breast cells from both irradiated and unirradiated cultures containing potential transformants were suspended at a concentration of 1 x 10^4/ml in medium containing 0.3% agar and 20% fetal bovine serum; each sample being plated over a 0.9% agar base layer and cells were examined for clonal growth at 21 days. Significant levels of anchorage independent growth were observed in assays of the human breast cells HME50 irradiated with total doses of 30,40, 50 (not shown) and 60 Gray, Figure 4B-D. These changes in anchorage-independent growth for the 30-60 Gray-treated cultures were reproducible in three separate determinations. In contrast, HME32 cultures from all treatment conditions (unirradiated and irradiated) tested negative anchorage-independent growth (data not shown). HME50 cells from the 10 and 20 Gray treated cultures showed only a small amount of anchorage-independent growth which was relatively inconsistent during the three separate determinations. In addition, all cultures from both cell lines were tested for adaptive growth in the presence serum. This was achieved in a step-wise manner by the inclusion of 2,5, and 10% fetal bovine serum in the culture medium at successive subcultivations of treated and untreated cultures of each cell line. The HME50 cells following treatment with 30-60 Grays of x-rays were able to sustain growth in the presence of serum (10% fetal bovine serum) and could proliferate in the absence of epidermal growth factor (EGF). In contrast, the unirradiated HME50 and 10-20 Gray treated HME50 cultures could not proliferate in the presence of serum and were still dependent for growth in the presence of bovine pituitary extract (BPE) and EGF. All the HME32 irradiated and unirradiated cultures were also dependent on the presence of BPE and EGF.

Tumorigenicity. The tumorigenic potential of the irradiated and unirradiated HME50 and HME32 cultures was determined. Three-week old female severe-combined-immune-deficiency *(scid)* mice were inoculated in the abdominal mammary fat-pad with 10^7 unirradiated human breast cells or radiation-induced transformants or 5 x 10^6 MCF-7 carcinoma cells (positive control), Table 1. Cells from the unirradiated, 10 and 20 Gray HME50 treated cultures were non-tumorigenic, that is they failed to form tumors in *(scid)* mice during six months. In contrast, cells from the 30-60 Gray treated HME50 cultures were tumorigenic with the highest incidence of tumor formation being seen in the 50 and 60 Gray treated HME50 cultures. These tumors developed within 8 weeks and were excised and processed for histopathology and re-established in culture. The tumors were diagnosed as moderately-differentiated adenocarcinomas. Cells re-established in culture were serially cultivated and tested again for their tumorigenic potential. Upon testing a second time, cells established from the tumors derived from the 40-60 Gray treated cultures were more tumorigenic (6/6) at 8 weeks in most cases. In contrast, all HME32 cultures, both irradiated and unirradiated controls were determined to be non-tumorigenic as the inoculated mice were monitored for six months.

TABLE 1

Total Dose	Saturation density cells per cm^2 x 10^{-5}	Soft agar colony formation*	Nude mice with tumors 10^7 cells**
None	2.1	<0.01	0/4
10 Gy (2 Gray x 5)	2.2	0.006	0/4
20 Gy (2 Gray x 10)	2.3	0.013	0/4
30 Gy (2 Gray x 15)	3.4	0.062	2/4
40 Gy (2 Gray x 20)	4.1	0.070	3/4
50 Gy (2 Gray x 25)	4.4	0.074	4/4
60 Gy (2 Gray x 30)	5.2	0.059	4/4

*Saturation density was measured as the maximum number of cells obtained after initial plating with 3 x 10^3 cells per cm^2 and then incubating at 37°C with growth media changes every 3 days.
**Three-week old female nude mice *(scid)* were inoculated in the abdominal mammary fat-pad with 10^7 unirradiated human breast cells or radiation-treated transformants or 5 x 10^6 positive control cells MCF-7 carcinoma cells. The MCF-7 positive controls were inoculated into overiectomized-estrogen supplemented female *scid* mice. Estrogen supplementation was by the use of 0.72 mg. 60 day-release pellets (Innovative Research, Fla. USA) implanted subcutaneously. The MCF-7 cells inoculated into mice gave rise to >1 centimeter tumors in all mice (6/6) after 12 weeks. The animals were examined twice each week for tumor formation. Tumors were then excised and (I) re-established in culture; (ii) processed for pathological analysis.

EXPERIMENTAL DESIGN and METHODOLOGY

Characterization and Culture of Human Breast Epithelial Cells.

The recent isolation of an immortalized human breast epithelial cell line HME50 from a patient with Li-Fraumeni's syndrome (103), and an additional cell line HME32 immortalized by the introduction of a mutant *p53* into normal mammary epithelial cells has provided means of testing the previously stated hypothesis. During the performance of these studies the Li-Fraumeni Syndrome (LFS) cell line (HME50) was grown in MEBM medium (Clonetics Corp

San Diego, CA.) supplemented with 0.4% bovine pituitary extract (BPE), 5ug insulin, 10ngs of epidermal growth factor (EGF), 0.5ug hydrocortisone and 5ug transferrin. These epithelial cells grown under these conditions express cytokeratins 14 (a basal cell marker), and cytokeratin 18 (a luminal cell marker). Breast epithelial cells obtained from milk appear to have more of a luminal cell type (expressing cytokeratin 19), as is the case for the majority of breast tumors, with only a subset showing evidence of basal markers (104). At this point in time there are no non-virally immortalized human breast cell lines available which express a keratin profile typical of a luminal phenotype, consequently the use of *p53* immortalized human breast epithelial cell lines e.g. HME50, that do not strictly express a luminal cytokeratin profile are the only ones available for this type of study. The second cell line we have used HME32, is a human breast epithelial line from a normal (p53 wild type) donor immortalized following transfection with an expression vector containing a mutant *p53* cDNA (containing a mutation at codon 273). Both of these cell lines do not exhibit anchorage-independent growth and are non-tumorigenic in athymic mice. These cell lines were a generous gift from Dr. J. Shay of University of Texas, Southwestern Medical Center, Dallas, Texas.

DNA sequence analysis of PCR generated fragments of the *p53* gene in the HME50 cells using primers flanking exon 5 has demonstrated the presence of a mutation at codon 133, resulting in an exchange of a methionine for a threonine leading to a conformational change in the wild-type *p53* protein (105,106). A similar DNA analysis of the donor's affected relatives has indicated that this mutation underlies the high frequency of early onset breast cancer in this family, with the incidence being traced back three generations (103). The conformational change induced by the mutation at codon 133 in this LFS breast cell line results in a loss of DNA binding activity and transcriptional activation by this protein (107). In contrast, the mutation at codon 273 does not result in a conformational change in the *p53* protein. This mutant *p53* protein retains the ability to bind to the *p53* consensus element and its transcriptional activation functions (108). It is entirely possible that these two immortalized human breast cell lines HME50 and HME32 will differ in their response to x-rays, either in their ability to undergo malignant transformation or radiation-induced cell cycle arrest.

Radiation treatment of human breast cells. Human breast cells were irradiated using a protocol similar to that used int he therapeutic treatment of breast carcinoma in situ. Exponentially growing cells were inoculated at 5 x 10^5 per 75 cm^2 flask and irradiated 24 hours later with 2Gy of gamma radiation using a JL Shepard Mark II Cesium 137 irradiation at a dose rate of 2.37Gy/min. Additional doses of 2Gy were delivered at daily intervals to a total dose of 60Gy. After each 10Gy increment, the cultures were allowed to recover for 7-10 days. After this recovery, a portion of the irradiated cultures were tested for changes in morphology, anchorage-independent growth, growth factor requirements (ie., removal of BPE, EGF or insulin), growth in presence of serum and tumorigenicity. The remaining cells were used for the additional radiation treatments to a total dose of 60Gy, outlined in Figure 2

Assay for anchorage independence. The development of the anchorage-independent phenotype following radiation treatment were determined by the method of McPherson (109). Potential transformants were suspended at a concentration of $1x10^4$/ml in medium containing 0.3% agar; each sample being plated over a 0.9% agar base layer. Cells were examined for clonal growth at 21 days; the number of colonies counted and expressed as the number of cell plated x 100 (colony-forming efficiency).

Tumorigenicity. Three-week old athymic female nude mice (scid) were inoculated in the abdominal mammary fatpad with 10^7 unirradiated human breast cells or radiation-induced transformants or positive control cells MCF7 carcinoma cells. The animals were examined twice each week for tumor formation. Tumors were then excised and (I) re-established in culture; (ii) processed for pathological analysis; and (iii) frozen in nitrogen for later analysis.

Statement of Work: Performance so far.

At the completion of these radiation studies we have produced changes in cellular morphology, saturation density at confluence and growth factor requirements in the HME50 cell line. In addition we have demonstrated that these irradiated HME50 cultures can develop both anchorage-independent and tumorigenic phenotypes. In contrast, the irradiated HME32 cultures (normal donor) did not produce any of these phenotypic changes in response to multiple doses of ionizing radiation. At this point in the these studies it will be necessary to isolate individual clones from all the radiation treated HME50 cell cultures and their tumorigenic derivatives as these cultures are most probably highly heterogeneous containing cells with numerous radiation-induced mutations. In order to perform the genetic studies described in Task1 of the Statement of Work (SOW) and all molecular studies outlined in Task2,(chromosomal analysis, SSCP analysis, wild-type p53 suppression of tumorigenic growth and cell cycle delay analysis), cellular clones from the radiation-transformed HME-50 cells along with clones we derived from the tumors developed in this study will be required to provide unequivocal and definitive answers to the previously stated hypothesis,

The possible answers will include: the remaining wild type *p53* allele in the HME-50 cells being mutated by either deletion resulting in the loss of expression of the *p53* protein or that additional point mutations have been introduced arising from a mis-incorporation during DNA repair. In the event of radiation inducing deletions in the remaining wild type allele this will result in the mutant form of the *p53* predominating resulting in a complete "gain in function." Alternatively, if additional point mutations have been introduced into the remaining *p53* allele, this will have the effect of increasing the gene dosage for mutant forms of *p53*. These additional point mutations will indicate an error in a DNA repair mechanism or the development of a state of genetic instability in the transformed cells.

Alternatively, it is possible that at the conclusion of these molecular studies the integrity and function of the remaining *p53* gene in the radiation-transformed breast cells remains unchanged. In this event other genetic mechanisms must be involved either through the activation of a proto-oncogene or the inactivation of a tumor suppressor gene. This will lead to future studies involving functional cloning of activated proto-oncogenes using DNA mediated gene transfer or expression cloning with cDNA libraries from the radiation-transformed breast cells. Alternatively, inactivated tumor suppressor genes involved can be identified using a positional cloning strategy. Both experimental approaches will aided by the prior evaluation of the chromosomal analysis.

Rationale for Incomplete Status of SOW: In our previous studies with human keratinocyte cultures we observed the development of morphological changes, anchorage-independent growth and tumorigenicity following just two treatments of 2 Gray (35). In contrast, the radiation-induced transformation of HME50 human breast epithelialcells required treatments to a minimum cumulative dose of 40 Grays. The cellular studies described in this report include radiation treatments, cell density studies, anchorage-independent determinations, assessment of growth factor requirements and tumorigenicity assays have been performed on both cell lines HME50- and HME32. Certain assays including saturation density and anchorage-dependency studies were performed three times on both cell lines at several stages during the radiation transformation studies. Further, the tumorigenicity studies were performed twice for each cell line. At the time of the original submission the number of radiation treatments required to develop the cellular changes described above and hence the length of time involved in performing these cellular studies was undetermined. In evaluating the progress of these studies so far it is clear that the project although well thought out in terms of the cellular and molecular biology and a testable hypothesis was extremely ambitious given the number of radiation treatments required to induced these phenotypic changes and the period of performance for the study (2 years). Realistically, an additional two years of study will be required to develop individual clones from the

radiation transformed human mammary epithelial cells (HME50) and perform the genetic and molecular analysis require to uniquivocally validate of refute the hypothesis as postulated

KEY ACCOMPLISHMENTS

We have demonstrated that the human breast epithelial cell line HME50, which is derived from a patient with Li-Fraumeni's syndrome can be morphologically transformed with successive doses of gamma radiation. In addition, these same radiation treatments of HME50 breast cells induce phenotypic changes, anchorage-independent growth and tumor formation in immunodeficient mice *(scid)*.

BACKGROUND TO AREA OF STUDY 'INTRODUCTION FROM ORIGINAL PROPOSAL'

The complexity of human breast cancer and the development of 130,000 new cases annually contrasts with our relative lack of knowledge of the biology of the disease (1). An improved understanding of this complex disease would be helped by determining the environmental agents involved in its development. Epidemiological studies have shown a role for a combination of factors, hormonal/reproductive history, diet, socioeconomic status, genetic predisposition, and the external factors such as ionizing radiation. Knowledge of the effects of ionizing radiation on the human mammary gland is important both for the estimation of environmental risk to human populations and for the study of normal tissues damage in the therapy of breast cancer. Although many epidemiological studies have shown a role for ionizing radiation in the development of human breast cancer, this tissue has remained unresolved. Ionizing radiations under appropriate conditions will induce cancers in experimental animals and humans and can act as complete carcinogens since they can both initiate and promote neoplastic transformation (2,3). This carcinogenic potential has proven controversial regarding diagnostic techniques and a potential deterrent to its use in radiation therapy. However, the cancer incidences of the atomic bomb survivors (4-6), and other studies of North American women exposed to medical irradiation (7-9), show that the breast is one of the most sensitive human tissues for radiation-induced carcinogenesis (10).

The recent cloning of the Ataxia telangiectasis gene has renewed interest in individuals with potential genetic predispositions for breast cancer (11). Individuals who are heterozygous for ataxia telangiectasia (-1% of the population) exhibit two significant characteristics: cancer predisposition and radiation sensitivity. Individuals who are homozygous for ataxia telangiectasia have an exceptionally high incidence of all cancers (12) and those who are heterozygous for this condition have an excess risk of cancer, particularly breast cancer and are considered a susceptible population (13). Cancer predisposition in this group has been estimated to be about three- to fourfold that of the general population, with a relative risk for breast cancer in carriers fivefold that of normal women (14-20). Among women in families affected with the hereditary disease Li-Fraumeni syndrome (LFS), breast tumors are the most prevalent cancer (affecting at least 50%) with 28% of the breast cancers being diagnosed before age 30 and 89% before age 50 (21-23). In spite of these observations ionizing radiation is routinely used in the therapy of primary breast cancer with many early tumors being treated conservatively by the surgical removal of the tumor followed by treatment of the remaining breast and associated tissues with radiation (17). The biological effects of such therapeutic doses of ionizing radiation at or near the location of surgery have not been fully established. As the use of this type of treatment increases, there will be an increasing incidence of radiation exposure of normal and benign breast tissues in patients (18). With the occurrence of new solid tumors being a well-recognized

consequence of therapeutic radiation (19,20), it will be important to determine the cellular and molecular effects of ionizing radiation on human breast cells.

Although ionizing radiations were one of the first proven environmental carcinogens (24,25), the molecular mechanisms of radiation carcinogenesis have remained poorly understood. Extensive studies using rodent cells in culture have developed quantitative relationships between dose, dose-rate, and quality of ionizing radiation with the eventual development of a tumorigenic phenotype (26-36). Initial molecular studies of radiation carcinogenesis have described the activation of cellular *ras* genes in rodent experimental systems (37). However, there are fewer studies describing the radiation-induced transformation of human cells and the molecular mechanisms involved (38-40). While rodent model systems employed in mammary cancer research have clarified certain areas of investigation, the known differences between human and rodent mammary physiology, in response to etiological agents, emphasizes the uncertainty in applying information gained in these model systems to the human situation. The direct study of human cells is the most appropriate way to determine the potential of etiological agents to initiate and promote human mammary neoplasia.

There are now several immortalized human breast cells from individual donors displaying a variety of changes which correlate with those observed during neoplastic development, e.g., extended lifespan, immortality, growth factor independence and tumorigenicity. While any of these immortalized cell lines cannot be considered to represent a normal phenotype, such cell lines with an indefinite lifespan are usually more amenable to experimentation than normal finite lifespan cells in determining the potential of chemical, physical carcinogens, oncogenic viruses or transfected genes, to induce malignant phenotypes.

The study of radiation-induced transformation of human cells has been hampered until the recent development of immortalized cell lines. Ionizing radiation as x-rays has been shown to extend the lifespan and immortalize normal embryonic human fibroblasts and epithelial cells (41-45). The development of a human epidermal keratinocyte cell line (RHEK) has provided a model system for studying human epithelial cell transformation (46,47). We have recently shown that these immortalized human keratinocytes can be transformed with x-rays into malignant cells (38). In a subsequent study we demonstrated that this radiation-induced transformation did not involve mutations or allelic losses in either the p53 tumor suppressor gene or the cellular *ras* genes (48). These studies and those performed by others used cells that were immortalized with viral oncogenes (SV40-T antigen or HPV16/18). Although such cells have proven useful in demonstrating malignant transformation of human cells with ionizing radiation, the presence of viral oncoprotein has made it difficult to evaluate the role of p53 tumor suppressor gene in radiation-induced malignant transformation of human cells. The availability of immortalized human breast epithelial cells not containing viral oncoproteins provided in vitro models to evaluate the role of p53. In a recent study by Wazer et.al. It was shown that normal human mammary epithelial cells can be transformed with 30 Gray of gamma-radiation to produce malignant cells (40). The amount of radiation used in this study was within the range of that used during conventional radiotherapy of breast cancer, typically 2 Gray daily fractions to a cumulative dose of 60-70 Gray. This malignant transformation was accompanied by the complete loss of p53 protein expression due to deletions in both alleles of the p53 gene. This study demonstrates the potential of the p53 gene as a target for radiation-induced mutations in human breast epithelial cells. Alterations in the p53 gene are commonly found in several types of human neoplasms including breast cancer (49,50) and in patients with tumors purportedly caused by radiation therapy (51). Further, patients with Li-Fraumeni syndrome who have heritable alterations in p53 are at an increased risk of developing certain cancers, including breast cancer, after radiation exposure (52). In transgenic mice, deletions in the p53 gene result in an increased sensitivity to radiation-induced tumorigenesis (53). These observations strongly implicate p53 as an important determinant in radiation carcinogenesis.

Ionizing radiation is known to induce DNA double-strand breaks which can lead to chromosomal deletions and rearrangements (54,55). Structural changes associated with the induction of mutations by ionizing radiation at autosomal loci in human cells, indicate that more than 70% of x-ray induced mutations involve the entire loss of an entire gene (56). Deletions in regions of DNA, indicating the loss of a tumor suppressor gene are also relatively common in breast cancer, usually been detected as a loss of heterozygosity in a polymorphic allele. Allelic loss for chromosome 13 has also been found in approximately 25% of breast carcinomas (57) which is the location of the chromosomal region containing the retinoblastoma-susceptibility gene. The highest frequency of allelic loss in primary breast carcinomas has been found for a region on the short arm of chromosome 17 (58). This region includes the *p53* gene.

The *p53* gene is a nuclear phosphoprotein which has been implicated in the control of normal proliferation and neoplastic transformation of cells (59,60). It is expressed at low levels in non-transformed cells but is often elevated in tumor-derived or transformed cell lines (60). Early studies showed that the *p53* gene could function as a dominant transforming oncogene (61-63), however, these studies employed mutated *p53* genes (60), and it has been shown that the wide-type gene is incapable of transformation (64-66). Further, expression of the wild type gene inhibits the activity of transforming genes in transfection assays (67), demonstrating that wild type *p53* is a suppressor of cellular growth. Evidence has been obtained for the functional suppression of the cellular growth of several different human cancer cell lines following DNA transfection or retro viral transfer of the wild-type *p53* gene (68-70). There is evidence that inactivating point mutations in the *p53* gene are involved in the etiology of many human cancers (71-76). Evidence is accumulating that mutations in the *p53* gene are important in the development of human breast cancer (72,77,78). Allelic losses have frequently been observed in the short arm of chromosome 17 in human breast tumors (79-81), consistent with the location of a tumor suppressor gene in this region. Although there may be more than one region of allelic loss on chromosome 17p (82), one is known to include the *p53* gene at 17p13 (82-85). Frequent over-expression of the *p53* gene has been reported in breast tumors (86) and there is a high correlation between elevated expression of the *p53* gene and loss of heterozygosity on the short arm of chromosomes 17 (87). Point mutations in the *p53* gene have been detected in both breast cancer cell lines and primary tumors (72,77,78,88,90) and abnormal histochemical staining using *p53* antibodies has been reported in approximately 50% of breast tumors examined (74,76-78,89).

Recently, two studies have shown germ line *p53* mutations in fibroblasts derived for both affected and non-symptomatic individuals exhibiting the hereditary cancer disease Li-Fraumeni syndrome (LFS) (21,90). Patients with this syndrome can develop variety of soft-tissue cancers, and breast cancer at an early age (22). A molecular explanation for the specifically increased incidence of breast cancer, particularly the early onset breast cancer, in families affected by LFS relative to other forms of cancer has not yet been elucidated (91-93). Taken together, these results strongly suggest that the wild-type *p53* gene may function as a suppressor of cellular growth in human breast cancer cells.

p53 is known to regulate cell cycle progression by modulating transcription and by interacting with cell cycle regulatory proteins (94-96). Recent studies have shown that normal *p53* protein is a cell cycle checkpoint determinant that controls the length of G1 phase to ensure an intact genome (97,98). Exposure to DNA-damaging agents, such as radiation, leads to an increase in *p53* levels followed by G1 arrest. Cells that lack wild-type *p53* protein fail to arrest in G1 following irradiation, and transfection of wild-type *p53* restores this response (98-99). These results have led to the hypothesis that *p53* is part of a protective mechanisms to prevent propagation of DNA damage. Loss of wild-type *p53* protein by deletion or mutation may allow for the accumulation of mutations that lead to aberrations in cellular growth control and eventual tumorigenesis. Consistent with this hypothesis,

epidemiological studies have shown an increased incidence of breast cancer in younger women who received diagnostic or therapeutic radiation for either breast cancer or other clinical disorders (100-102).

REFERENCES

1. **Russo J, Tay LK, and Russo IH:** Differentiation of the mammary gland and susceptibility to carcinogenesis. Breast Cancer Res Treat 2:5-73, (1982).
2. **Pitot HC:** Principles in Cancer Biology, Chemical Carcinogenesis. Lippincott, Philadelphia, PA, (1989).
3. **Little JB:** Cancer Etiology: Ionizing Radiation. Lea and Febiger, Malvern, PA, (1991).
4. **Wanebo CK, Johnson KG, Sato K and Thorsulund TW:** Breast cancer after exposure to the atomic bombings of Hiroshima and Nagasaki. N Engl J Med 279:667-671, (1968).
5. **Tokunaga ML, and CE, Yamamoto T, Asano M, Tokuoka S, Ezaki H and Nishimori I:** Breast cancer among atomic bomb survivors. Lancer II: 924, (1982).
6. **Tokuka S, Asano M, Yamamoto T, Tokunaga M, Sakamoto G, Hartmann WH, Hutter RV, Land CE and Henson DE:** Histologic review of breast cancer in survivors of atomic bombs of Hiroshima and Nagasaki. Jpn Cancer 54:849-854, (1984).
7. **Boice JD and Monson R:** Breast cancer in women after repeated fluoroscopic examinations of the chest. J Natl Cancer Inst 59:823-832, (1977).
8. **MacKenzie I:** Breast cancer following multiple fluoroscopies. Br J Cancer 19:1-8, (1965).
9. **Mettler FA, Hempelmann LH, Dutton AM, Pifer JW, Totooka ET and Ames WR:** Breast neoplasms in women treated with x-rays for acute postpartum mastitis-A pilot study. J Natl Cancer Inst 43:803-811, (1969).
10. **Committee on the Biological Effects of Ionizing Radiation.** Natl Acad Sci USA. 326, (1980).
11. **Sativitsky K, et.al.** A single Ataxia telangiectasia gene with a product similar to PI-3 kinases. Science 268, p1749-1753, (1995).
12. **Spector BD, et.al.** Epidemiology of cancer in ataxia telangiectasia. **In:** Ataxia Telangiectasia: A cellular and molecular link between cancer, neuropathology and immunodeficiency; eds: Bridges BA.\, Harden DG, Chichester, England. John Wiley 103-138, (1982).
13. **Swift M, Reitenauer PJ, Morrel D and Chase CL:** Breast and other cancers in families with ataxia telangiectasia. N Engl J Med 316:1289-1294, (1987).
14. **Bridges BA and Arlett CF:** Risk of breast cancer in ataxia telangiectasia. N Engl J Med 326:1357, (1992).
15. **Boice JD, Jr. and Miller RW:** Risk of breast cancer in ataxia telangiectasia. N Engl J Med 326:1357, (1992).
16. **Hall EJ, Geard CR and Brenner DJ:** Risk of breast cancer in ataxia telangiectasia. N Engl J Med 326:1358, (1992).
17. **Clark RM, Wilkinson RH, Mahoney LJ, Reid JG and MacDonald WD:** Breast cancer: A 21-year experience with conservative surgery and radiation. Int J Rad Oncol Biol Phys 8:267, (1982).
18. **Mattsson A, Ruden BI, Hall P, Wilking N and Rutqvist LE:** Radiation-induced breast cancer: Long term follow-up of radiation therapy for benign breast disease. J Natl Cancer Inst 85:1679-1685, (1993).
19. **Li FP, Cassady R and Jaffe N:** Risk of second tumors in survivors of childhood cancer. Cancer 35:1230-1235, (1975).
20. **Tucker MA, Coleman CN, Cox RS, Varghese A and Rosenberg SA:** Risks of second cancers after treatment of Hodgkin's disease. N Engl J Med 318:76-81, (1988).

21. **Malkin D, Li FP, Strong LC, Fraumeni JF, Nelson CE, Kim DH, Kassel J, Gryka M, Bischoff FZ, Tainsky MA and Freind SH:** Germ line *p53* mutations in a familial syndrome of breast cancer, sarcomas and other neoplasms. Science 250 p1233-1238, (1990).
22. **Li FP and Fraumeni JF:** Soft tissue sarcomas, breast cancer and other neoplasms. A familial syndrome? Ann Intrn Med 71 p747-751, (1969).
23. **Hartley AL, Birch JM, Marsden HB and Harris M:** Breast cancer risk in mothers of children with osteosarcoma and chondrosarcoma. Br J Cancer 54:819-823, (1986).
24. **Muller H:** Artificial transmutation of the gene. Science 66:84, (1927).
25. **Sigematsu L and Kagan A:** Cancer in atomic bomb survivors, GANN Monograph on Cancer Research 32 Tokyo: Japan Scientific Societies Press and New York: Plenum Press. (1986).
26. **Todaro GJ and Green H:** Quantitative studies on the growth of mouse embryo cells in culture and their development into established cell lines. J Cell Biol 17:299-313, (1963).
27. **Reznikoff CA, Brankow DW and Heidelberger C:** Establishment and characterization of a cloned line of C3H mouse embryo cells sensitive to postconfluence inhibition of cell division. Cancer Res 33:3231-3238, (1973).
28. **Reznikoff CA, Bertram JS, Brankow DW and Heidelberber C:** Quantitative and qualitative studies of chemical transformation of cloned C3H mouse embryo cells sensitive to postconfluence inhibition of division. Cancer Res 33:3239-3249, (1973).
29. **Borek C, Pain C and Mason H:** Neoplastic transformation of hamster embryo cells irradiated in utero and assayed in vitro transformation by x-irradiation. Nature 266:452-454, (1977).
30. **Borek C and Sachs L:** In vitro cell transformation by x-irradiation. Nature 210: 276-278, (1966).
31. **Borek C:** Neoplastic transformation following split doses of x-rays. Br J Rad 50:845-846, (1979).
32. **Borek C and Hall EJ:** Transformation of mammalian cells in vitro by low doses of x-rays. Nature 243:450-453.
33. **Borek C and Hall EJ:** Effect of split doses of bx-rays on neoplastic transformation of single cells. Nature 252:499-501, (1974).
34. **Borek C, Hall EJ and Rossi HH:** Malignant transformation in culture hamster embryo cells produced by x-rays, 430 KeV monoenergetic neutrons, and heavy ions. Cancer Res 39:2997-3005, (1978).
35. **Han A and Elkind MM:** Transformation of mouse C3H/10T1/2 cells by single and fractionated doses of x-rays and fission spectrum neutrons. Cancer Res 39:123-130, (1979).
36. **Han A and Elkind MM:** Enhanced transformation of mouse C3H/10T1/2 cells by 12-o-tetradecanoyl-phorbol-13-acetate following exposure to x-rays or to fission-spectrum neutrons. Cancer Res 42:477-483, (1982).
37. **Guerrero I, Villasante A, Corces V and Pellicer A:** Activation of a c-K-ras oncogene by somatic mutation in mouse lymphomas induced by gamma radiation. Science 255:1159-1162, (1984).
38. **Thraves P, Salehi Z, Dritschilo A and Rhim JS:** Neoplastic transformation of immortalized human epidermal keratinocytes by ionizing radiation. Proc Natl Acad Sci USA 87:1174-1177, (1990).
39. **Hei TK, Piao CQ, Willey JC, Thomas S and Hall EJ:** Malignant transformation of human bronchial epithelial cells by radon-simulated alpha-particles. Carcinogenesis 15:431-437, (1994).
40. **Wazer D, Chu Q, Liu X, Gao Q, Safaii H and Band V:** Loss of *p53* protein during radiation transformation of primary human mammary epithelial cells. Mol Cell Biol 14:2468-2478, (1994).
41. **Tuynder M, Godfrine S, Cornelis JJ and Rommelaere J:** Dose-dependent induction of resistance to terminal differentiation in x-irradiated cultures of normal human keratinocytes. Proc Natl Acad Sci USA 88:2638-2642, (1991).

42. **Kano W and Little JB:** Mechanisms of human cell neoplastic transformation: X-ray induced abnormal clone formation in long-term cultures of human fibroblasts. Cancer Res 45:2550-2555, (1985).
43. **Kano Y and Little JB:** Mechanisms of human cell neoplastic transformation: Relationship to specific abnormal clone formation to prolonged lifespan in x-irradiated human diploid fibroblasts. Int J Cancer 36:407-413, (1985).
44. **Namba M, Nishitani K, Hyodoh F, Fukushima F and Kimoto T:** Multistep process of neoplastic transformation of normal human fibroblasts by 60Co gamma rays and Harvey sarcoma viruses. Int J Cancer 35:275-280, (1985).
45. **Namba M, Nishitani K, Fukushima F, Kimoto T and Nose K:** Neoplastic transformation of human diploid fibroblasts (KMST-6) by treatment with 60Co gamma rays. Int J Cancer 37:419-423, (1986).
46. **Rhim J, Jay G, Arstein P, Price FM, Sanford KK and Aaronson SA:** Neoplastic transformation of human epidermal keratinocytes by AD12-SV40 virus and Kirsten sarcoma viruses. Science 227:1250-1252, (1985).
47. **Rhim J, Fujita J, Arnstein P and Aaronson SA:** Neoplastic conversion of human epidermal keratinocytes by AD12-SV40 virus and chemical carcinogens. Science 232:385-288, (1986).
48. **Varghese S, Jung M, Prasad S, Rhim JS, Dritschilo A and Thraves P:** Role of *p53* and ras genes in radiation-induced transformation of immortalized human epidermal keratinocytes. Rad Oncol Investigations 2:119-125, (1994).
49. **Nigro JM et al:** Mutations in the *p53* gene occur in diverse tumor types. Nature (Lond.) 342:705-708, (1989).
50. **Osborne RJ, Merlo GR, Mitsudomi T, Venesio T, Liscia DS, Cappa APM, Chiba I, Takahashi M, Nau M, Callahan R and Minna JD:** Mutations in the *p53* gene in primary human breast cancers. Cancer Res. 51:6194-6198, (1991).
51. **Brachman DG, Hallahan DE, Beckett DE, Beckett MA, Yandell DW and Weichselbaum RR:** *p53* mutations and abnormal retinoblastoma protein in radiation-induced human sarcomas. Cancer Res. 51:6393-6396, (1991).
52. **Srivastava S,Zou ZQ, Pirrollo K, and Chang ES:** Germline transmission of a mutated gene in a cancer prone family with Li-Fraumeni syndrome. Nature (Lond.) 348:747-749, (1990).
53. **Kemp CJ, et al:** *p53* deficient mice are extremely susceptible to radiation-induced tumorigenesis. Nat. Genet. 8:66-69, (1994).
54. **Upton AC, Albert RE, Burns EJ, Shore RE (eds).** Radiation carcinogenesis, Elsevier Science Publishing, Inc., New York, (1986).
55. **Wallace SS.** Environ Mutagen 5:769-788, (1983).
56. **Yuasa Y. Kamiyama T, Kato M, Iwama T, Ikeuchi T, Tonomura A:** Transforming genes from familial edematous polyposis patient cells detected by tumorigenicity assay. Oncogene 5:589-596, (1990).
57. **Lundberg C, Skoog L, Cavenee WK and Nordenskjold M:** Loss of heterozygosity in human ductal breast tumors indicates a recessive mutation on chromosome 13. Proc Natl Acad Sci USA 84:2372-2376, (1987).
58. **Sato T, Tangami A, Yamakawa K, Akiyama F, Kasumi F, Sakamoto G and Nakamura Y:** Allotype of breast cancer: Cumulative allele losses promote tumor progression in primary breast tumors indicates a recessive mutation on chromosome 13. Cancer Res 50:5184-7189, (1990).
59. **Lane DP and Crawford LV.** T-antigen is bound to a host protein in SV40 transformed cells. Nature 278 p261-263, (1979).
60. **Lane DP and Benchimol A.** p53: oncogene or anti-oncogene? Genes Dev. 4 pl-8 (1990).
61. **Eliyahu D, Raz A, Gruss P, Givol D and Oren M:** Participation of *p53* cellular antigen in transformation of normal embryonic cells. Nature 312 p646-649, (1984).

62. **Jenkins JR, Rudge K and Currie GA:** Cellular immortalization by a cDNA clone encoding the transformation-associated phosphoprotein *p53*. Nature 312 p651-654, (1984).
63. **Parada LF, Land H, Weinberg RA, Wolf D and Rotter V:** Cooperation between gene encoding *p53* tumor antigen and ras in cellular transformation. Nature 312 p649-651, (1984).
64. **Eliyahu D, Goldfinger N, Pinhasi-Kimhi O, Shaulsky G, Skurnik Y, Arai N, Rotter V and Oren M:** Meth A fibrosarcoma cells express two transforming mutant *p53* species. Oncogene 3 p313-321, (1988).
65. **Finlay CA, Hinds PW, Tan TH, Eliyahu D, Oren M and Levine AJ:** Activating mutations for transformation by *p53* produce a gene product that forms an hsc70-*p53* complex with an altered half-life. Mol. Cell. Biol. 8 p531-539, (1988).
66. **Hinds P, Finlay C and Levine AJ:** Mutation is required to activate the *p53* gene for cooperation with the ras oncogene and transformation. J. Virology. 63 p739-746, (1989).
67. **Finlay CA, Hinds PW and Levine AJ:** The *p53* proto-oncogene can also act as a suppressor of transformation. Cell 57 p1083-1093, (1989).
68. **Baker SJ, Markowitz S, Fearon ER, Willson JK and Vogelstein B:** Suppression of human colorectal carcinoma cell growth by wild-type *p53*. Science 249 p912-915, (1990).
69. **Diller, Kassel J, Nelson C, Gryka M, Litwak G, Gebhardt M, Bressac B, Ozturk M, Baker SJ, Vogelstein B and Friend SH:** *p53* functions as a cell cycle control protein in osteosarcomas. Mol Cell Biol 10 p5772-5781, (1990).
70. **Mercer WE, Shields MT, Amin M, Sauve G, Appella E, Romano JW and Ullrich SJ:** Negative growth regulation in a glioblastoma tumor cell line that conditionally expresses human wild-type *p53*. Proc Natl Acad Sci. USA 87 p6166-6170, (1990).
71. **Baker SJ, Fearon ER, Nigro JM, Hamilton SR, Preisinger AC, Jessup JM, van Tuinen D, Ledbetter DH, Barker DF, Nakamura Y, White R and Vogelstein B:** Chromosome 17 deletions and *p53* gene mutation in colorectal carcinomas. Science 244 p217-221, (1989).
72. **Nigro JM, Baker S, Preisinger AC, Jessup JM, Hostetter R, Cleary K, Bigner S, Davidson N, Baylin S, Devilee P, Glover T, Collins FS, Weston A, Modali R, Harris CC and Vogelstein B:** Mutations in the *p53* gene occur in diverse human tuor types. Nature 342 p705-708, (1989).
73. **Takahashi T, Nau MM, Chiba I, Birrer MJ, Rosenberg RK, Vincour M, Levitt M, Pass H, Gazdar AF and Minna J:** *p53* .A frequent target for genetic abnormalities in lung cancer. Science 246 p491-494, (1989).
74. **Iggo R, Gatter K, Bartek J, Lane D and Harris AL:** Increased expression of mutant forms of *p53* oncogene in primary lung cancer. Lancet 335 p675-679, (1990).
75. **Mulligan LM, Matalashewski GJ, Scable HJ and Cavenee W:** Mechanisms of *p53* loss in human sarcomas. Proc Natl Acad Sci. USA. 87 p5863-5867, (1990).
76. **Rodrigues NR, Rowan A, Smith MEF, Kerr IB, Bodmer WF, Gannon JV and Lane DP:** *p53* mutations in colorectal cancer. Proc Natl Acad Sci. USA. 87 p7555-7559, (1990).
77. **Bartek J, Bartkova J, Vojtesek B, Staskova Z, Rejthar A, Kovarik J and Lane DP:** Patterns of expression of the *p53* tumor suppressor gene in human breast tissues and tumors *in situ.* Int J Cancer 46 p839-844, (1990).
78. **Bartek J, Iggo R, Gannon J and Lane DP:** Genetic and immunohistochemical analysis of mutant *p53* in human breast cancaer cell lines. Oncogene 5 p893-899, (1990).
79. **Mackay J, Steel CM, Elder PA, Forrest APM and Evans HJ:** Allelic loss of short arm of chromosome 17 in breast cancers. Lancet II p1384-1385, (1988).

80. **Devilee P, van den Broek M, Kuipers-Dijkdhoorn N, Kollurui R, Khan PM, Pearson PL and Cornelisse CJ:** At least four different chromosomal regions are involved in the loss of constitutional heterozygosity in human breast carcinoma. Genomics 5 p554-560, (1989).
81. **Varley JM, Brammar WJ, Lane DP, Swallow JE, Dolan C and Walker RA:** Loss of chromosome 17p13 sequences and mutation of *p53* in human breast carcinomas. Oncogene 5 p413-421, (1991).
82. **Coles C, Thompson AM, Elder PA, Cohen BB, Mackensie TM, Cranston G, Chetty U, Mackay J, MacDonald M, Nakamura Y, et al:** Evidence implicating at least two genes on chromosome 17 in breast carcinogenesis. Lancet 336:761-763, (1990).
83. **Devilee P, Cornelisse CJ, Kuioers-Dijkshoorn J, Jonker C and Pearson PL:** Loss of heterozygosity in human breast carcinomas, defining the smallest common region of deletion. Cytogenet Cell Genet 53 p52-54, (1990).
84. **Isobe M, Emanuel BS, Givol D, Oren M and Croce CM:** Localization of gene for human *p53* tumor antigen to band 17p13. Nature 320 p84-85, (1986).
85. **McBride OW, Merry D and Givol D:** The gene or human *p53* cellular tumor antigen is located on chromosome 17 short arm 17p13. Proc natl Acad Sci. USA. 83 p130-134, (1986).
86. **Crawford LV et al.** The cellular protein *p53* in human tumors. Mol Cell Med. 2 p261-272, (1984).
87. **Thompson AM, Steel CM, Chetty U, Hawkins RA, Miller WR, Carter DC, Forrest APM and Evans HJ:** *p53* gene mRNA expression and chromosome 17p allele loss in breast cancer. Br J Cancer e61 p74-78, (1990).
88. **Prosser J, Thompson AM, Cranston G and Evans HJ:** Evidence that *p53* behaves as a tumor suppressor gene in sporadic breast tumors. Oncogene 5 p1573-1579, (1990).
89. **Cattoretti G, Rilke F, Andreola S, D'Amato L and Delia D:** *p53* expression in breast cancer. Int J Cancer 41 p179-183, (1988).
90. **Srivastava S, Zou Z, Pirillo K, Blattner W and Chang ES:** Germline transmission of a mutated *p53* gene in a cancer prone family with Li-Faumeni syndrome. Nature 348 p747-749, (1990).
91. **Li FP, Fraumeni JF, Mulvhill JJ, blattner W, Dreyfus MG, Tucker MA and Miller RW:** A cancer family syndrome in twenty-four kindreds. Cancer Res 48:5358-5362, (1988).
92. **Harris JP et al:** Breast cancer. N Engl J Med . 327:49-53, (1992).
93. **Osteen RT and Karnell KH:** The National Cancer Data Base report on breast cancer. Cancer 73:1994-2000, (1994).
94. **Bischoff JR, Freidman PN, Marsak DR, Prives C and Beach D:** Human *p53* is phosphorylated by p60-cdc2 and cyclinB-cdc2. Proc Natl Acad Sci. USa. 87 p4766-4770, (1990).
95. **Funk WD, Pak DT, Karas RH, Wright WE and Shay JW:** A transcriptionally active DNA binding site for human *p53* protein complexes. Mol Cell Biol. 12:p2866-2871, (1992).
96. **Sturzbecher HW, Maimets T, Chumakov P, Brain R, Addison C, Simanis V, Rudge K, Philip R, Grimaldi M, Court W, et al:** *p53* interacts with p34/cdc2 in mammalian cells: implications for cell cycle control oncogenesis. Oncogene 6 p775-781, (1990).
97. **Kastan MB, Onyekwere O, Sidransky D, Vogelstein B and Craig RW:** Participation of *p53* protein in the cellular response to DNA damage. Cancer Res. 51 p6304-6311, (1991).
98. **Kuerbitz SJ, Plunkett BS, Walsh WV and Kastan MB:** Wild-type *p53* is a cell cycle checkpoint determinant following irradiation. Proc Natl Acad Sci. USsa. 89 p7491-7495, (1992).
99. **Kessis TD, Slebos RJ, Nelson WG, Kastan MB, Plunkett BS, Han SM, Lorincz A, Hedrick L and Cho KR:** Human papillomavirus 16 E6 expression disrupts the *p53*-mediated cellular response to DNA damage. Proc Natl Acad Sci. USA 90:3988-3992, (1993).

100. **Boice JD Jr, Harvey EB, Blettner M, Stovall M and Flannery JT:** Cancer in the contralateral breast after radiotherapy for breast cancer. New Eng J Med. 326 p781-785, (1992).
101. **Boice JD Jr, Preston D, Davis FG and Monson RR et al:** Frequent chest x-ray fluoroscopy and breast cancer incidence among tuberculosis patients in Massachusetts. Radiat Res. 125 p214-222, (1991).
102. **Hancock SLM, Tucker MA and Hoppe RT:** Breast cancer after treatment of Hodgkins disease. Int J Cancer. 85 p25-31, (1993).
103. **Shay JW, Tomlinson G, Piatyszek MA and Gollhahon LS:** Spontaneous in vitro immortalization of breast epithelial cells from a patient with Li-Fraumeni syndrome. Mol Cell Biol. 15:425-432, (1995).
104. **Taylor-Papadimitriou J, Berdichevski F, D'Souza B and Burchell J:** Human models of breast cancer. Cancer Surv. 16:59-78, (1993).
105. **Law JC, Stong LC, Chidambaram A and Ferrell RE:** A germline mutation in exon 5 of the *p53* gene in an extended family. Cancer Res 51:6385-6387, (1991).
106. **Srivastava S, Wang S, Tong YA, Pirrollo K and Chang ES:** Several mutant *p53* detected in cancer prone families with Li-Fraumeni syndrome exhibit transdominant effects on the biochemical properties of wild-type *p53*. Oncogene 8:2449-2456, (1993).
107. **Srivastava S et al:** Several mutant *p53* proteins detected in cancer-prone families with Li-Fraumeni syndrome exhibit transdominant effects on the biochemical properties of the wild-type *p53*. Oncogene 8:2449-2456, (1993).
108. **Zhang W, Funk WD, Wright WE, Shay JW and Deisseroth AB:** Novel DNA binding of *p53* mutants and their role in transcriptional activation. Oncogene 8:2555-2559, (1993).
109. **MacPherson I.** Agar suspension culture for quantitation of transformed cells. In Habel, K and Salzman, NP (eds), Fundamental Techniques in Virology. Academic Press, New York p214-219, (1969).
110. **Wolf D, Harris N and Rotter V:** Reconstitution of *p53* expression in a non-producer Ab-MuLV-transformed cell line by transfection of a functional *p53* gene. Cell 38:387-394, (1984).
111. **Levine AJ, Monmand J and Finlay C:** The *p53* tumor suppressor gene. Nature 351:453-456, (1991).
112. **Oren M.** *p53;* the ultimate tumor suppressor gene ? FASEB 6:3169-3176, (1992).
113. **Milner J and Medcalf EA:** Cotranslation of activated mutant *p53* with wild type drives the wild type *p53* protein into the mutant conformation. Cell 65:765-774, (1991).
114. **Bargonetti J et al:** Site specific binding of wild type *p53* to cellular DNA is inhibited by SV40 T-antigen and mutant *p53*. Genes Dev. 159:529-534 (1986).
115. **Kern S, Pientenpol JA, Thiagalingam S, Seymour A, Kinzler KW and Vogelstein B et al:** Oncogenic forms of *p53* inhibit *p53*-regulated gene expression. Science 256:827-830, (1992).
116. **Milner J and Medcalf EA:** Temparature-dependent switching between wild-type and mutant *p53* forms of *p53*-val. J Mol Biol 216:481-484, (1990).
117. **Sturzbecher HW, Chumakov P, Welch WJ, and Jenkins JR:** Mutant *p53* binds hsp 72/73 cellular heat shock proteins in SV40-transformed monkey cells. Oncogene 1:201-211, (1987).
118. **Kraiss S, Quaiser A, Oren M, and Montenarh M:** Oligomerization of oncoprotein *p53*. J Virol. 62:4737-4744, (1988).
119. **Milner J, Medcalf EA and Cook AC:** The tumor suppressor *p53*: analysis of wild type and mutant *p53* complexes. Mol Cell Biol 11:12-19, (1991).
120. **Orita M, Iwakana H, Hagashi K and Sekuya T:** Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. Proc Natl Acad Sci. USA. 86:2766-2770, (1989).

121. **Murakami Y, Hayashi K, and Sekiya T:** Detection of abberations of the *p53* alleles and the gene transcript in human tumor cell lines by single-strand conformation polymorphism analysis. Cancer Res 51: p3356, (1991).
122. **Thraves P, Varghese S, Jung M, Grdina D, Rhin DJ, Rhin JS and Dritschilo A:** Transformation of human epidermal keratinocytes with fission neutrons. Carcinogenesis 15:2867-2873, (1994).
123. **Nakamura Y, Ballard L, Leppert M, O'Connel P, Lathrop GM, Lalouel JM and White R:** Isolation and mapping of a polymorphic DNA sequence (pYNZ22) on chromosome 17[D17S30]. Nucleic Acid Res 16: p1507, (1988).
124. **Yuxin Y, Tainsky M, Biscoff FZ, Stong LC and Wahl G:** Wild-type *p53* restores cell cycle control and inhibits gene amplification in cells with mutant *p53* alleles. Cell 70:937-948, (1992).
125. **Preston-martin S, Pike M, Ross RK, Jones PA and Henderson BE:** Increased cell division as a cause of human cancer. Cancer Res. 50:7415-7421, (1990).
126. **Cohen SM and Ellwein LB:** Cell proliferation and carcinogenesis. Science 249:1007-1111, (1990).
127. **Kastan MB et al:** A mammalian cell-cycle checkpoint pathway utilizing *p53* and gadd45 is defective in ataxia telangietasia. Cell 71:587-597, (1992).
128. **Fornace AJ, Nerbert DW, Hollader C, Luetin JD, Papathanasiou M, Fargnoli J and Holbrook NJ:** Mammalian genes coordinately regulated by growth arrest signals and DNA damaging agents. Mol Cell Biol. 9:4196-4203, (1989).
129. **El-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer E, Kinzler KW and Vogelstein B:** WAF1, a potential mediator of *p53* tumor suppression. Cell 75:817-825, (1993).
130. **Smith ML, Chen IT, Zhang Q, Bae I, Chen CY, Gilmer TM, Kastan MB, O'Connor PM and Fornace AJ Jr:** Interaction of the *p53*-regulated protein gadd45 with proliferating cell nuclear antigen. Science (Washington, DC). 266:1376-1380, (1994).
131. **Harper JW, Adami GR, Wei N, Keyomarsi K and Elledge SJ:** The p21 Cdk-interacting protein Cipl is a potent inhibitor of G1 cyclin-dependent kinases. Cell 75:805-816, (1993).
132. **Chen J, Jackson PK, Kirschner MW and Dutta A:** Separate domains of p21 involved in the inhibition of Cdk-kinase and PCNA. Nature (Lond.), 374:386-388, (1995).
133. **Clarke AR, Purdie CA, Harrison DJ, Morris RG, Bird CC, Hooper ML and Wyllie AH:** Thymocyte apoptosis induced by *p53* dependent and independent pathways. Nature 362:849-852, (1993).
134. **Lowe SW, Schitt EM, Smith SW, Osbourne BA and Jacks T:** *p53* is required for radiation-induced apoptosis in mouse thymocytes. Nature 360:847-849, (1993).
135. **Nagasawa H, Li CY, Maki CG, Imrich AC and Little J:** Relationship between radiation-induced G1 phase arrest and *p53* function in human tumor cells. Cancer Res. 1842-1846, (1995).
136. **Little JB, Nagasawa H, Keng PC, Yu Y and Li CY:** Absence of radiation-induced G1 arrest in two closely related human lymphoblast cell lines that differ in *p53* status. J Biol Chem 270:11033-11036, (1995).
137. **Petersen WD Jr, Simpson WF, and Hukku B:** Cell culture characterizing:monitoring for cell identification. Methods Enzymol. 58:164-178, (1979).
138. **Petersen WD Jr, Ottenbreit MJ and Hukku B:** Uses and standardization of vertebrate cultures. In Levine, E.M. et al (eds); In Vitro Monograph, vol 5:116-124, (1984)
139. **Puck TT, Marcus PI.** Action of x-rays on mammalian cells. Journal of Experimental Medicine. 103:653-660, 1956.
140. **Elkind MM, Sutton H.** X-ray damage and recovery in mammalian cells in culture. Nature, 184:1293-1295, 1959.
141. **Elkind MM, Sutton H.** Radiation response of mammalian cells grown in culture. Radiat. Res., 13:556-693, 1960.

142. **Albright N.** Computer programs for the analysis of cellular survival data. Radiat. Res., 112:331-340, 1987.

FIGURE LEGENDS

Figure 1. Radiation toxicity Studies: Human breast epithelial cells (HME-50) were irradiated with graded doses (0-11 Gray) of gamma-rays and radiation survival determined by colony formation assay (139), for a minimum of three independent determinations. Survival data obtained from these experiments were fitted using a computer program, Albright et al (142) using a least-squares method, to determine the a and b values for a linear-quadratic model and n and Do for a multi-hit and single-target model according to the method of Elkind and Sutton (140,141).

Figure 2. Radiation Transformation Scheme. Exponentially growing cells were inoculated at 5 x 10^5 per 75 cm^2 flask and irradiated 24 hours later with 2 Gray of gamma radiation using a JL Shepard Mark II Cesium 137 irradiator at a dose rate of 2.37 Gray/min. Additional doses of 2Gray were delivered at daily intervals to a total dose of 60 Gray. After each cumulative 10 Gray increment, the cultures were allowed to recover for 7-10 days, Figure . After this recovery, a portion of the irradiated cultures were tested for changes in morphology, anchorage-independent growth, growth factor requirements (i.e. removal of BPE, EGF or insulin), growth in presence of serum and tumorigenicity. The remaining cells were used for the additional radiation treatments to a total dose of 60 Gray.

Figure 3. Human breast epithelial cells were treated with 2 Gray fractions of gamma-rays followed by growth to confluence with nutrient medium and photographed (13.2x mag.). **A.** Unirradiated HME-50 cells., **B.** Unirradiated HME-50 cells, (66 x mag.). HME-50 cells irradiated with: **C.** 20 Gray (10 x 2 Gray), **D.** 30 Gray (15 x 2 Gray), **E.** 40 Gray (20 x 2 Gray), and **F.** 60 Gray (30 x 2 Gray).

Figure 4. Anchorage-independent growth of irradiated HME-50 cells. Growth in 0.33% soft agar for cultures treated with 2 Gray fractions of gamma-rays and unirradiated controls were scored at 21 days. **A.** Unirradiated HME-50 cells., **B.** HME-50 cells treated with 30 Gray (15 x 2 Gray), **C.** 40 Gray (20 x 2 Gray) and **D.** 60 Gray (30 x 2 Gray).

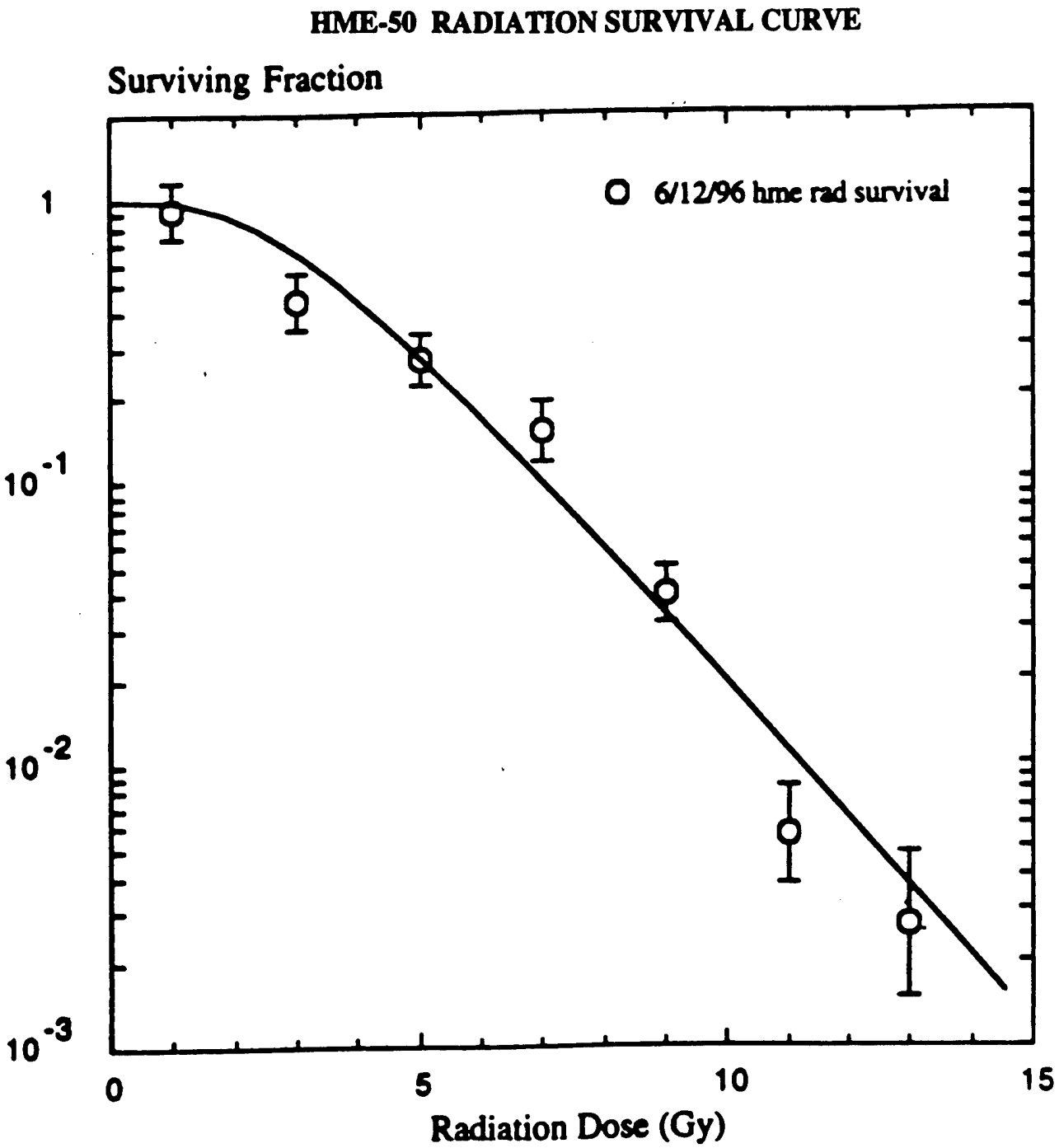

HME-50 RADIATION SURVIVAL CURVE
Surviving Fraction
1
10^{-1}
10^{-2}
10^{-3}
O 6/12/96 hme rad survival
0
5
10
15
Radiation Dose (Gy)

Figure 1

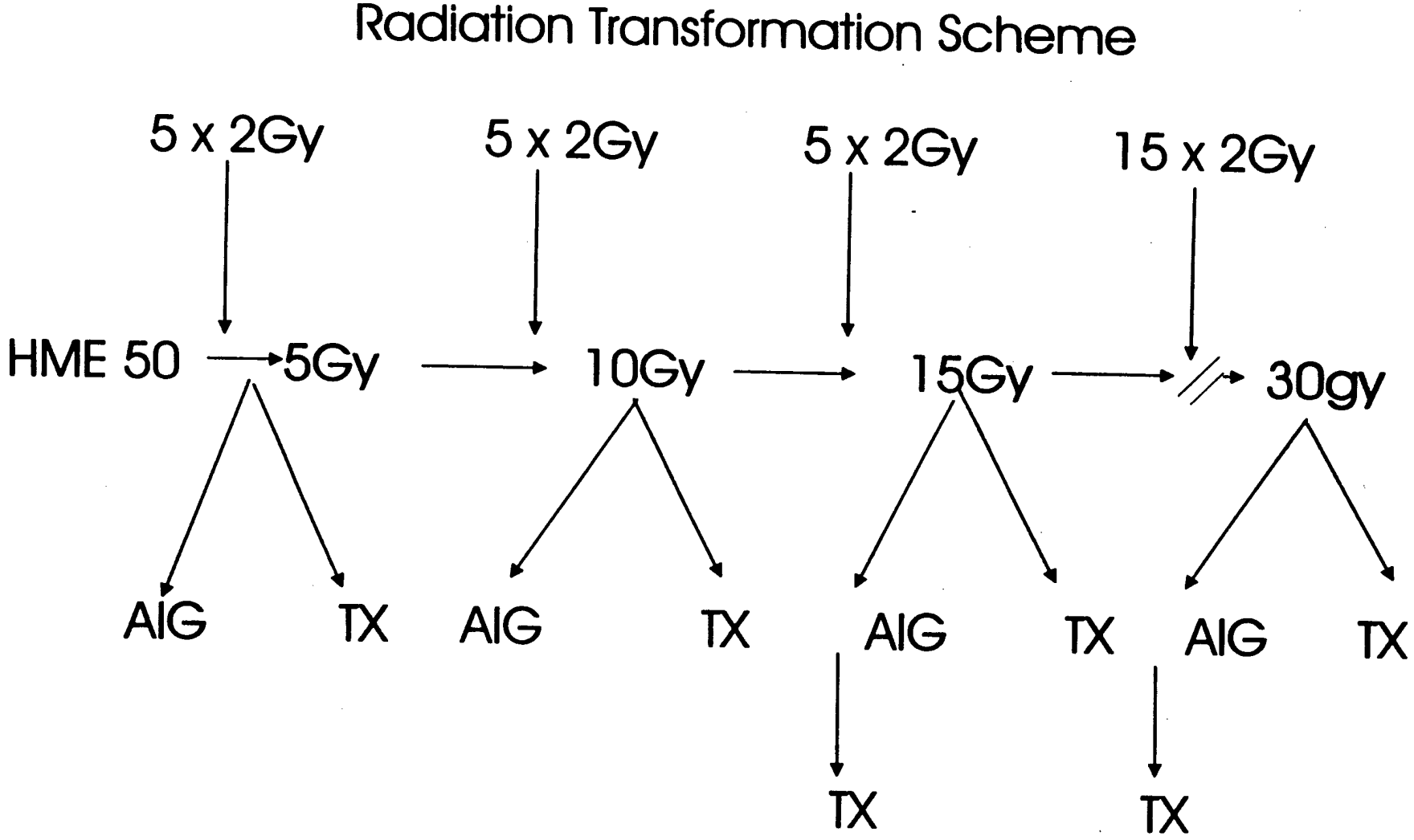


Figure 2

AIG: Anchorage-independent growth assay
TX: Tumorigenicity testing

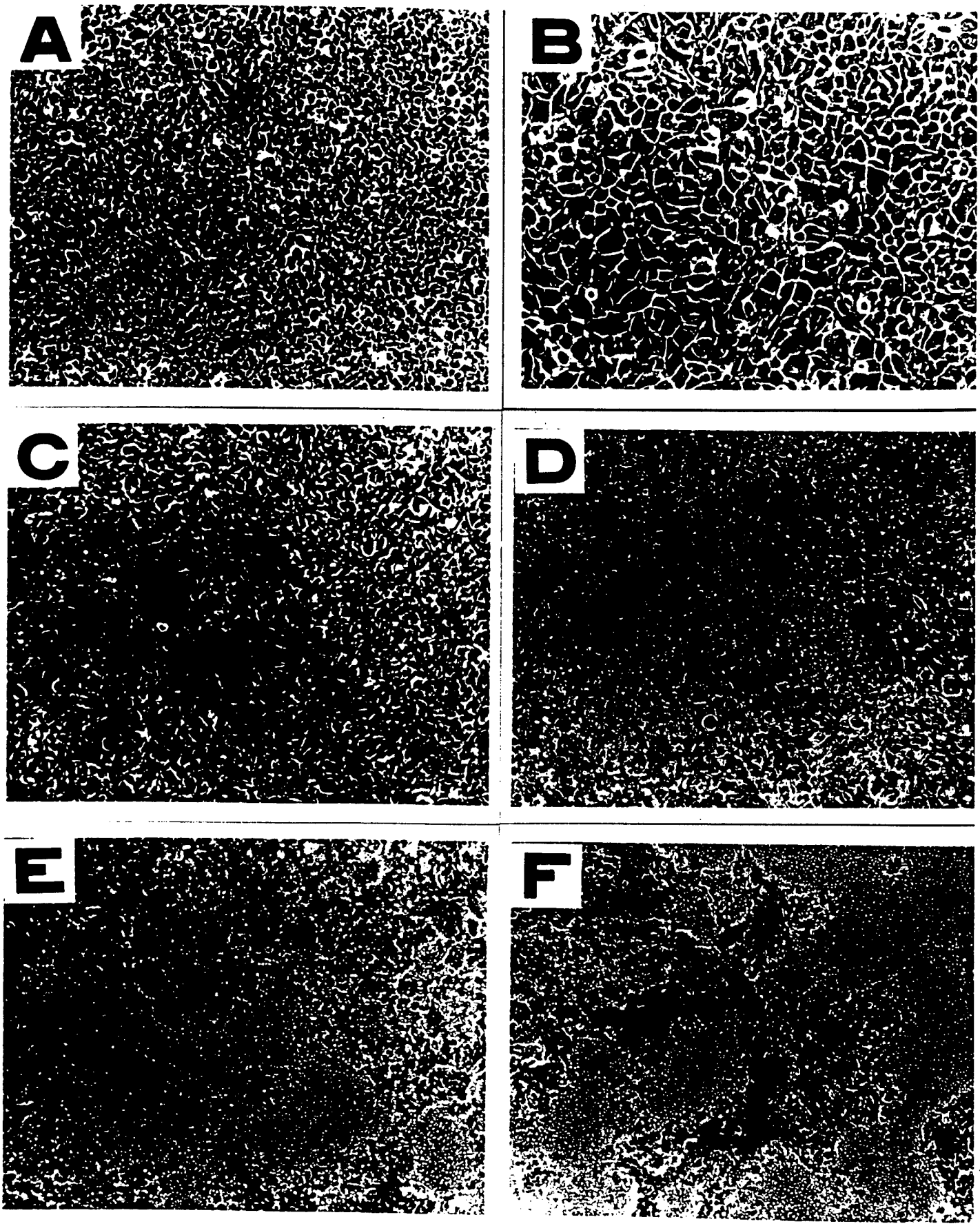
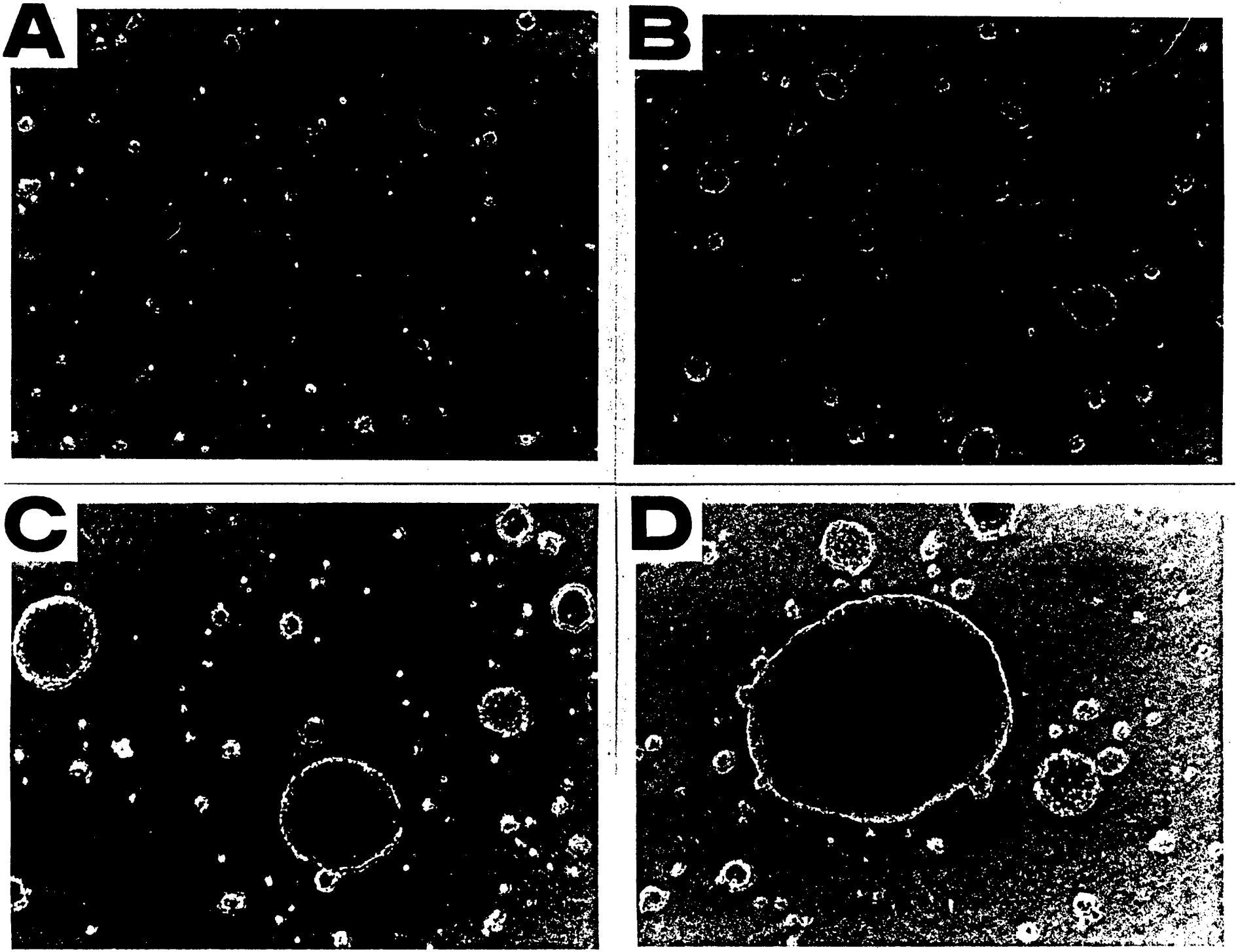


Figure 3


A
B
C
D